REMARKS

Claims 19-44 are now pending in the present application, claims 1-18 having been canceled and new claims 19-44 added. All of the new claims are drawn to methods that fall within the restriction group presently under examination.

Support for the new claims can be found in the original claims and throughout the specification. For example, support for new claims 19, 20, 21 and 22 can be found in original claim 15; in the specification at page 3, lines 26-34; page 10, lines 34-36; page 11, lines 1-12; and page 12, lines 8-11; and in Figures 1 and 2. Support for new claims 23-26 and 27-30 can be found in original claim 16 and in the specification at page 10, lines 34-36; page 11, lines 1-12; page 12, lines 8-11; page 17, lines 26-36; and page 18, lines 1-3 and lines 18-20. Support for new claims 31-34 can be found in original claims 12 and 15 and in the specification at page 12, lines 22-25. Support for new claims 35-38 and 39-42 can be found in original claims 8, 12 and 16. Support for new claims 43 and 44 can be found in original claim 17 and in the specification at page 18, lines 6-17. No new matter has been added.

Applicant requests entry of the above amendment and allowance of the new claims in view of the remarks in this Response.

Information Disclosure Statement

The Office action at pages 2-3 indicates that the information disclosure statements (IDS) filed on December 17, 2004, and September 15, 2005, failed to comply with 37 CFR 1.98(a)2, which requires the submission of a legible copy of each cited reference, and with 37 CFR 1.98(a)1, which requires listing of the application number for which the IDS is being submitted.

Applicant respectfully points out that the Office erred in entering the September 15, 2005 IDS into this file. This IDS is clearly intended for a different application, as indicated by the facts that both the attorney docket number (14875-148US1) and application filing date

(December 13, 2005) are not the same as those of the present application (attorney docket number 14875-134US1 and filing date of June 21, 2005). Applicant requests that the Examiner delete the IDS of September 15, 2005, IDS from this file.

Copies of the references cited in the IPER for the corresponding PCT application were not submitted with the IDS of December 17, 2004, because it is the Applicant's understanding that such materials are routinely made available to the Office by WIPO and so duplicates need not be submitted by Applicant. Nonetheless, Applicant has submitted copies of the references with this response for the Examiner's convenience.

Specification

The Office action at pages 3-4 suggests that the "Brief Description of the Drawings" be moved from page 21 to a location consistent with the guidelines in 37 CFR 1.77(b). Accordingly, the above amendment to the specification relocates the "Brief Description of the Drawings" to page 6, and also revises one section heading to read "Summary of the Invention" and adds a new section heading "Detailed Description".

35 U.S.C. §112, second paragraph

The Examiner rejected claims 15-17 for alleged indefiniteness. Without conceding that the claims as previously presented fail to satisfy this requirement, and solely for the purpose of furthering prosecution, claims 15-17 have been cancelled, and thus the rejection as applied to these claims is moot. As discussed below, Applicant believes that the grounds for the rejections would not apply to the newly presented claims.

1) Claims 15-17 were rejected as being indefinite for omitting the essential steps of "preparing or introducing an expression vector" (Office Action at page 4). The rejection was apparently based on the fact that these claims were drawn to "A method for constructing an antibody library or expression vector..." but did not mention expression vectors in the body of the claims. None of the present independent claims recites "expression vector" in its preamble or in any step of the

claim, and none requires use of an expression vector (though they of course do no exclude such an embodiment, and in fact dependent claims 32, 34, 36 and 40 specify that an expression vector is involved). Thus, Applicant submits that the claims as presently amended do not lack any essential step.

2) Claims 15-17 were rejected for the recitation of the term "long linker." (Office Action at page 4). That term no longer appears in any of the claims.

3) Claim 15 was rejected for the recitation "the other ends comprise a restriction enzyme site" because it was "not clear whether the other ends refers to the unlinked ends for either one or both of the VH domain and VL region." (Office Action at page 5). The new claims clearly specify that both the sequence encoding the VH domain and the sequence encoding the VL domain (or, in some claims, the sequences encoding the "third variable domain" and the "fourth variable domain") have an end (distal to the linker) with a specified restriction site.

4) Claim 15 was rejected for insufficient antecedent basis for the recitation "the fragments obtained from the above treatment" (Office Action at page 5). The new claims do not contain that language and provide antecedent basis for all terms.

5) Claim 15 was rejected for insufficient antecedent basis for the recitation "the heavy and light chain variable domains against the second antigen" because "element b) recites 'a light chain variable *region*' directed against 'a second antigen'" (Office Action at page 5). The new claims consistently use the term "domain", not "region".

6) Claim 16 was rejected for insufficient antecedent basis for the recitation "the gene" in steps a and c (Office Action at page 5). The new claims do not use that term.

7) Claims 15-17 were rejected as indefinite for the recitation "constructing an antibody phage library in which a light chain variable domain and a heavy chain variable domain...restriction enzyme sites" because "it is not clear how only one VL and VH domain can comprise a library,

when a plurality of VL/VH pairings would seemingly be required to comprise the library" (Office Action at page 5). Applicant points out that the quoted language of claims 15-17 did not say that there is only one VL and one VH domain represented in the entire library, and those claims were not so limited as the Examiner supposes. To make the intended scope even clearer, new claims 19, 21, 23, 27, and 43 say that "each member of the library [comprises] a nucleotide sequence encoding a light chain variable domain and a nucleotide sequence encoding a heavy chain variable domain," or something to that effect. This language plainly permits variation among the members of the library.

8) Claim 17 was rejected for insufficient antecedent basis for the recitation "the fragments obtained above" in step c (Office Action at page 5). This language does not appear in the new claims.

9) Claim 17 was rejected as indefinite for the recitation "both against an antigen" because "it is not clear if the VH and VL domains should bind the same or different antigen" (Office Action at page 6). None of the new claims uses that language.

Applicant submits that all of the rejections for indefiniteness have been overcome. Withdrawal of the rejections is requested.

35 U.S.C. §103

Claims 15-17 stand rejected as unpatentably obvious in view of McGuinness et al. (Nat. Biotech. 14: 1149-1154 (1996)) and Völkel (Protein Engineering 14(10): 815-823 (2001)). According to the Office Action at pages 7-8:

> McGuiness discloses methods for constructing an antibody phage display library where the V regions from antibodies against the hapten phOx or Dig are constructed into two pools of scFvs repertoires having a 15-amino acid linker between each VH and VL domain, where the orientation of the domains is VH-linker-VL (p. 1150, Col.1, ¶1). The scFV pools were recombined into a diabody format: VHA-VLB-rbs (linker)-VHB-VLA, where the linker between each VH and VL domain was "shortened" to a zero linker (p. 1150, Col. 2, ¶ 1) using one

> of two methods: ligation mediated assembly or cassette cloning where the final diabody is inserted into an expression vector.

The Office Action continues:

> The 15 amino acid linker of McGuiness is considered as reading on the linker for the first and second single scFVs of Claim 1 and the HV and LV of Claim 17. The claims are not drawn to the specific order in which the VH1 and VL1 or the VH2 and VL2 should occur. In other words, McGuiness teaches a diabody format: VHA-VLB-rbs (linker)-VHB-VLA which reads on the instant claims. The method steps of subcloning fragments and digesting the fragments with enzymes to arrive at the diabody structure is not excluded by claims 15-17, therefore, the steps of McGuinness read on the claims.
>
> Volkel discloses constructing a diabody phage display library comprising single chain diabody CEA scFV/Gal scFv with a randomized middle linker from where the M linker is of variable length and comprises at least one restriction site.....Volkel discloses generating a fragment comprising GalVL-M linker-GalVH where the M linker comprises a restriction site and subcloning the fragment in the linker region for the CEA scFv where the linker region comprises two restriction enzyme sites, BstE II and Sac I. (Office Action at pages 8-9.)

Applicant respectfully traverses this rejection. As discussed below, the Office has failed to establish that all the claim limitations are found in the cited art.

New claims 19-42 claim methods of constructing libraries (or constructs) encoding *single chain* diabodies, that is, where all four variable domains of the diabody are expressed as part of a single polypeptide. This was implied in claims 15 and 16 as originally presented (see, *e.g.*, the final step of each of those claims). Those claims are now canceled and replaced with new claims 19-42, which make the "single chain" aspect explicit in their preambles as well as in their final ligation steps.

Contrary to the Examiner's apparent reading of McGuinness, and in distinct contrast to the presently claimed methods, McGuinness does not disclose such a method. Rather, McGuinness discloses a method of preparing a library in which a single construct encodes two separate scFv chains that are, after expression, capable of assembling into a two-chain diabody. See, for example, the description of McGuinness' construct at page 1150, right column:

> The format used for these diabody constructs (including vector based features) is (5' to 3'): ribosome binding site (rbs)-Leader 1-V_HA-V_LB-**Stop**-rbs-Leader 2-V_HB-V_LA-His_6-Myc-Amber codon-fd gene 3 (Fig.1).

This description of McGuinness' constructs makes it clear that they encode two separate polypeptides: note the mention of a "Stop" codon following the V_LB, as well as the two ribosome binding sites and the two leader sequences, necessarily implying that two distinct polypeptides are expressed from the construct. This is further confirmed by the statement at page 1152, right column, that "each clone produces two polypeptide chains." Thus, the Examiner's assumption (quoted above) that McGuinness' "diabody format... reads on the instant claims" is simply wrong. McGuinness teaches nothing whatsoever about single-chain diabody formats, whether applicant's or any other.

Nor do McGuinness's methods of making his constructs bear any significant resemblance to applicant's methods. For example, McGuinness does not disclose use of a first antibody library in which the sequences encoding the heavy and light chain variable domains are linked by a nucleotide linker containing a restriction site, as required by step (a) of claim 19. It follows that this reference also does not disclose a step of cleaving at that restriction site, as required by step (c). Furthermore, McGuinness does not disclose inserting a sequence encoding a second pair of heavy and light chain variable domains into the open restriction site, and certainly does not disclose generating a construct encoding a single polypeptide chain comprising all four variable domains, all as required by step (e). In fact, it is difficult to derive from McGuinness any teaching of relevance to the presently claimed invention.

Unlike McGuinness, Volkel does disclose constructs encoding single-chain diabodies. However, any similarity to applicant's library methods pretty much ends there. Volkel started with a known single chain diabody construct containing four variable domains (pAB1 scDb CEAGal), not a pair of libraries, each member of which encodes two variable domains, as required by steps (a) and (b) of claim 19. Thus, none of the subsequent steps of the claims, all of which require manipulations of those libraries, was carried out by Volkel. As

Volkel was interested in determining the effect of linker length on function of the diabody, Volkel used several restriction enzyme sites located in the pAB1 scDb CEAGal construct's three linkers as a means to generate a library of constructs that varied solely in the lengths of the three linkers (page 816, columns 1-2; fig.2). Volkel did not ligate a nucleic acid fragment encoding two variable domains into a cleaved restriction site between two variable domains, as required by step (e) of claim 19, and provided no reason to contemplate doing so. (In fact, introducing extra variable domains into her construct in that manner would have ruined her experiment, the purpose of which was simply to study the effect of linker length.) Accordingly, Volkel does not disclose any of the steps of the present claims. Since Volkel was not concerned with combining two two-variable-domain libraries to prepare a four-variable-domain, single chain diabody library, and in fact started out with a construct that already contained all four variable domains in a single chain, Volkel provides no incentive to make the considerable modifications that would be required to result in a method such as applicant's. Volkel certainly does not supply what is absent from McGuinness, and vice versa.

The above discussion focused on claim 19, for simplicity. However, the same considerations apply to independent claims 21, 23, 27, 31, 33, 35 and 39 as well. All of these claims involve generation of nucleic acids that encode single chain diabodies (either as a library or as a construct), so the teachings of McGuinness are equally irrelevant to all of them. All start with a first library (or first nucleic acid) encoding two variable domains and a second library (or second nucleic acid) encoding two variable domains, and specify a method for manipulating the libraries/nucleic acids and then ligating the members of the two libraries (or the two nucleic acids) together in a very particular way. Volkel teaches nothing at all like the claimed methods, and no reason to seek to accomplish what applicant has accomplished. There is no apparent reason to combine the teachings of the two references, and even if one were to combine the two references, it would not result in a method that includes any of the steps of claims 19-42.

Claim 17, which was rejected *en bloc* along with claims 15 and 16 as obvious in view of the combination of McGuinness and Volkel, actually presents different issues than do the other independent claims discussed above. Claim 17 has been canceled and replaced with new claims 43-44, which encompass the same general subject matter as claim 17.

Claim 43 is drawn to a method that begins with an antibody library in which each member of the library comprises a first and a second nucleotide sequence, each sequence encoding a variable domain, the two nucleotide sequences being connected to each other by a linker of 30 to 150 base pairs comprising two or more cleavage sites for a restriction enzyme. After treating the library with the restriction enzyme to cleave the two or more sites within the linker, the cleaved product is self-ligated to generate a second antibody library in which the sequences encoding the variable domains are joined by a nucleotide linker that is shorter than the linker in the original library. The claimed method provides a convenient way to shorten the peptide linker that joins the two variable domains encoded by each member of the library. Such a method would be useful, for example, if it is desired to make the peptide linker too short to permit intrachain interaction between the two variable domains, thereby facilitating interchain interactions and thus dimerization to form two-chain diabodies. See, e.g., the specification at pages 2-3, carryover paragraph.

Neither McGuinness nor Volkel, nor the two in combination, discloses such a method. McGuinness describes libraries in which a single nucleic acid encodes two separate scFv, each scFv containing two variable domains joined by a peptide linker. McGuinness did not describe any reason one would want to start with a long linker and then shorten it, and certainly did not disclose the presently claimed method of including two or more cleavage sites in the sequence encoding the linker, then using a restriction enzyme to cleave those sites, followed by self-ligation of the cleaved product to close up the gap created by the restriction enzyme digestion.

These deficiencies are not compensated by Volkel. Volkel studied how varying the length and sequence of each of the three linkers (labeled A, M (for "middle"), and B) in a four-variable-domain single chain diabody would affect the function of the single chain diabody.

Volkel started with a single, defined sc diabody construct containing four defined variable domains, arranged in the order VH1-VL2—VH2-VL1 (see Fig.2). As detailed on page 816, col.1-2 (carryover paragraph), treatment with different restriction enzymes and PCR amplification with primers of various lengths and sequences yielded eight different fragments that were mixed and ligated together in either one or four ligation reactions, to yield a library of constructs all encoding the original set of just four variable domains, but with different middle linkers (M). Then various positive clones isolated from that library were digested with two other restriction enzymes and amplified with various primers of different lengths and sequences in order to introduce diversity in the length and sequence of the other two linkers (A and B). The resulting library was screened for ability to bind to the sc diabody's two cognate antigens.

Thus, Volkel did not disclose a method that is anything like the claim 43 method. There are at least four glaring differences. *First*, Volkel started with a single, defined construct, not an antibody library as required by the claim. *Second*, when Volkel cleaved the linker between the sequences encoding the VL2 and VH2 (i.e., the middle linker), she did not then self-anneal the cleaved product, as required by the claim, but rather carried out PCR amplification with several different primers and then annealed the resulting mixture of PCR-amplified products. *Third*, even if one were to equate the defined construct of Volkel to the library of step (a) of the claim, the "linker M" library produced by annealing Volkel's PCR-amplified products did not meet the limitation "each member of the second library comprising the first and the second nucleotide sequences joined by a linker than is shorter than the linker in the library of (a)". As shown in Table III on page 818, Volkel's mixture produced a library of sc diabodies varying by middle linker length; some of these were shorter than the original 16-amino acid (i.e., 48 bp) "wt" middle linker, but others were longer: specifically, 17 or 18 amino acids. *Fourth*, each of the other two linkers in Volkel's construct, the so-called "A" and "B" linkers, connected a heavy chain variable domain and a light chain variable domain that were not directed against the "same antigen," as required by claim 43; rather, one variable domain was directed against carcinoembryonic antigen (CEA) and the other against β galactosidase (see Fig. 2). Only linker "M" linked two variable domains directed against the "same" antigen (β galactosidase), as

required by the claim. Accordingly, Volkel's manipulations of the A and B linkers also do not meet the criteria of claim 43.

Thus, for several reasons, Volkel's methods are quite different from that claimed in claim 43. Neither Volkel nor McGuinness provides any motivation to modify the methods of either reference to come anywhere near that presently claimed.

The Examiner has not established that McGuinness and Volkel, either alone or in combination, teach or suggest *all the limitations of the subject matter now claimed.* Nor do these references provide any motivation to modify each others' teachings to arrive at the presently claimed inventions. They disclose entirely different experiments done for entirely different reasons. In view of the foregoing, the Office is respectfully asked to reconsider and withdraw this ground for rejection.

Applicant submits that the claims are now in condition for allowance, and such action is requested. A petition for extension of time is being filed herewith. Please charge any required fees to Deposit Account 06-1050, referencing attorney docket no. 14875-134US1.

Respectfully submitted,

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